Structural Studies of the I-PpoI homing endonuclease

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INTRODUCTION

'Homing' is the lateral transfer of an intervening genetic sequence, either an intron or an intein, to a cognate allele that lacks that element [1-5]. The end result of homing is the duplication of the intervening sequence. The process is initiated by site-specific endonucleases that are encoded by open reading frames within the mobile elements. Several features of these proteins make them attractive subjects for structural and functional studies. First, these endonucleases, while unique, may be contrasted with a variety of enzymes involved in nucleic acid strand breakage and rearrangement, particularly restriction endonucleases. Second, because they are encoded within the intervening sequence, there are interesting limitations on the position and length of their open reading frames, and therefore on their structures. Third, these enzymes display a unique strategy of flexible recognition of very long DNA target sites. This strategy allows these sequences to minimize non-specific cleavage within the host genome, while maximizing the ability of the endonuclease to cleave closely related variants of the homing site. Recent studies explain a great deal about the biochemical and genetic mechanisms of homing, and also about the structure and function of several representative members of the homing endonuclease families.

STUDIES CONDUCTED AT THE ADVANCED LIGHT SOURCE AND FHCRC

Conformational changes and DNA target site bending Induced by the His-Cys box homing endonuclease I-PpoI. The homing endonuclease I-*Ppo*I displays a highly extended fold and loosely packed central dimer interface, yet it DNA target site is severely bent in the protein-bound complex, resulting in sharp deformation of minor and major groove dimensions near the scissile phosphates [6]. In order to study the relative role of protein and DNA conformational changes during binding, we have determined the structure of the enzyme in the absence of bound DNA to 2.0 Å resolution, performed a fluorescence transfer analysis of the DNA homing site bending in the presence and absence of bound protein, and have mutagenized a leucine residue that plays a critical role in DNA bending. The structure of this enzyme point mutant has been determined bound to the DNA homing site before and after cleavage to 2.7 Å resolution, and the effect of this mutation on binding affinity and turnover velocity has been measured. The structure of the apo-enzyme displays a small rigid-body rotation of dimer subunits compared to the DNA-bound structure, and the homing site is not significantly bent in the absence of protein, indicating that binding involves a large distortion of the DNA onto a moderately static protein surface. The structure of the L116A enzyme mutant bound to DNA is almost identical to the wild-type apo-enzyme, while the affinity of this complex is reduced by at least 1000-fold. The large energetic cost of binding for this mutated enzyme is reflected in a large decrease in turnover rate. This study provides a direct example of the importance of binding energy for catalytic rate-enhancement.

A novel endonuclease mechanism directly visualizedby intermediate trapping. The structure of the I-*Ppo*I endonuclease complexed with its target DNA substrate has been determined in three distinct states on the cleavage pathway: bound in the absence of divalent cations, bound in the presence of magnesium prior to cleavage, and bound to cleaved product with magnesium. Each of these states was trapped by two separate strategies for comparison of the structures. The resulting structures demonstrate that this enzyme follows a single metal mechanism in which the nucleophilic water is activated by a conserved histidine residue, and the bound metal (which is coordinated by a single conserved asparagine residue and two contacts to the DNA) acts to stabilize the phosphoanion transition state and the 3' hydroxyl leaving group. These structures also demonstrate conformational motions of the active site side chains and the scissile phosphate that accompany cleavage, and indicate that a perturbed, high energy enzyme-substrate-metal complex is formed that is relieved upon cleavage. This mechanism appears to be reproduced in a non-specific nuclease and in the T4 endonuclease VII enzyme [7].

ACKNOWLEDGMENTS

Thanks to Thomas Earnest at the ALS for facilitating these studies and assisting in data collection.

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This work was supported by the National Insitutes of Health, under Contract No. GM49857.

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